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# Current Status Review
# The nuclear retinoid receptors

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It has been known for many years that vitamin A, or retinol, is required in the visual process and that retinol deficiency can result in blindness. More recently it has become clear that metabolites of retinol, such as those shown in Figure 1, have a role as signalling molecules that regulate cell behaviour during embryonic development and in adult life. This activity is thought to underlie both the teratogenic effects of retinoid excess in humans (Lammer *et al.* 1985) and the efficacy of retinoids as drugs for the treatment of some chronic dermatoses and certain forms of cancer (Darmon 1991; Martin *et al.* 1992). Retinoids exert their effects on cells by means of a complex signal transduction system. The purpose of this article is to describe our present understanding of this system and to review recent data indicating that elements of it may themselves be involved in the aetiology of certain malignancies.

## The origin, storage and mobilization of vitamin A

Vitamin A is obtained from plant carotenoids and animal retinyl esters in the diet and is stored predominantly in the form of retinyl esters in liver stellate cells. Retinol is mobilized into plasma as a complex with retinol-binding protein and delivered to a range of target tissues. A number of interstitial retinoid-binding proteins have been identified in extracellular spaces and these may be involved in regulating the delivery of retinoids to cells (for a review see Blomhoff *et al.* 1990). Once inside the target cell, retinol can be metabolized to all-*trans*-retinoic acid, 9-*cis*-retinoic acid, 3,4-didehydroretinoic acid (Figure 1) and no doubt to a number of other biologically active retinoids (Thaller & Eichele 1990).

There are a number of intracellular proteins with which retinoids can interact specifically. These are the cellular retinol-binding proteins (CRBP-I and CRBP-II), the cellular retinoic acid-binding proteins (CRABP-I and CRABP-II) and the nuclear retinoid receptors. Each of the CRBPs and the CRABPs is expressed in a tissue-specific fashion. Their functions are not entirely clear. However, there is evidence that CRBP-I may serve to concentrate retinol in cells with a requirement for its metabolites and that CRABP-I may act as a sink that regulates the concentration of free intracellular retinoic acid available to interact with nuclear receptors (Boylan & Gudas 1991). It is thought that these molecules may also play a direct role in regulating retinoid catabolism within target cells. It is not the purpose of this article to discuss these proteins in detail (for a review see Blomhoff *et al*, 1990).

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## The RAR and RXR classes of nuclear retinoid receptor

Retinoids act by binding to retinoid receptors in the nucleus of the cell. These proteins belong to the steroid/thyroid hormone nuclear receptor superfamily and share with them a modular structure of six domains, designated A to F (Figure 2a). Binding of retinoic acid to the E domain converts the receptor into an active transcription factor that is able to regulate transcription from gene promoters containing a specific nucleotide sequence, termed a retinoic acid response element (RARE). Dimerized receptors bind to RAREs via their C domains (Figure 2b).

Two classes of nuclear retinoid receptors have been identified in vertebrates (Table 1). These are the retinoic acid receptors (RARs) and the retinoid X receptors (RXRs). There are three distinct RAR genes in humans, termed RAR-$\alpha$ (Petkovich *et al.* 1987), RAR-$\beta$ (Brand *et al.* 1988) and RAR-$\gamma$ (Krust *et al.* 1989). Highly conserved homologues of all three genes have been identified in mice (Zelent *et al.* 1989), chickens (for a review see Rowe *et al.* 1992) and amphibians (Ragsdale *et al.* 1989; Ellinger-Ziegelbauer & Dreyer 1991). The predicted amino acid sequences of RAR-$\alpha$, RAR-$\beta$ and RAR-$\gamma$, which indicate relative molecular masses for the proteins of approximately 50 000, are very similar in the B, C, D and E domains but have no significant similarity in the

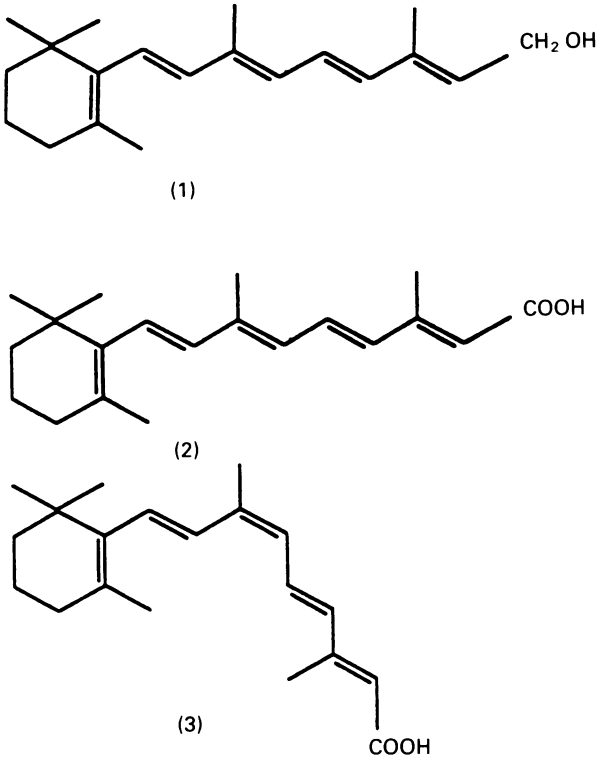


**Figure 1.** (1) Retinol and some of its naturally occurring metabolites: (2) all-*trans*-retinoic acid; (3) 9-*cis*-retinoic acid; (4) 3,4-didehydroretinoic acid.

A and F domains. There are also three RXR genes in humans, rodents and chickens, termed RXR-α, RXR-β and RXR-γ (Mangelsdorf *et al.* 1990; 1992; Yu *et al.* 1991; Rowe *et al.* 1991; Leid *et al.* 1992). The predicted amino acid sequences of RXR-α, RXR-β and RXR-γ are very similar in the B, C, D and E domains but not in the A and F domains. The DNA-binding C domain is highly conserved between the RAR and RXR classes, but there is no significant amino acid identity between the other domains of the RARs and the RXRs.

## Multiple RAR and RXR gene products

Analysis of cDNA clones and of the mRNAs from which

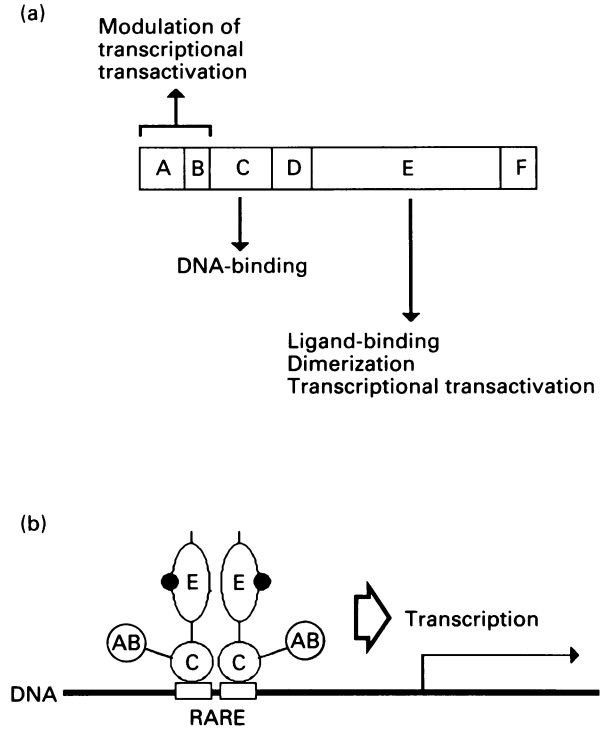


**Figure 2.** a, General domain structure of the retinoid nuclear receptor proteins. b, Activation of transcription by a retinoid nuclear receptor dimer, from a promoter containing a retinoic acid response element (RARE). ●, Ligand.

they are derived indicates that the RAR-α, RAR-β, and RAR-γ genes each encode more than one protein (Table 1; for a review see Leroy *et al.* 1992). The murine RAR-α gene appears to encode at least two protein isoforms (mRAR-α1 and mRAR-α2), which differ only in their N-terminal A domains. These proteins are encoded by two

**Table 1.** Nuclear retinoid receptors in vertebrates

| Gene | Protein products* | Chromosomal localization of human gene | General features of expression pattern in the mouse | |
|---|---|---|---|---|
| | | | embryo | adult |
| RAR-α | α1, α2 | 17q21.1† | w | w, blood cells |
| RAR-β | β1, β2, β3, β4 | 3p24‡ | r | r |
| RAR-γ | γ1, γ2 | 12q13§ | r | r, skin |
| RXR-α | α1 | 9¶ | w | w |
| RXR-β | β1, β2 | 6¶ | w | w |
| RXR-γ | γ1, γ2 | np | r | r |

*Identified to date in human, mouse and/or chicken. †Petkovich *et al.* (1987). ‡Brand *et al.* (1988). §Krust *et al.* (1989). ¶Mangelsdorf and Evans (1992). np, Not published; w, widespread; r, highly restricted.

different mRAR-α mRNAs that arise as a result of transcription from two distinct mRAR-α gene promoters (Leroy *et al.* 1991a, 1991b). The murine RAR-β gene appears to encode at least four protein isoforms (mRAR-β1, mRAR-β2, mRAR-β3 and mRAR-β4), which also differ only in their A domains. These are encoded by four different mRNAs that are generated by the use of two different promoters and by alternative splicing of primary transcripts (Zelent *et al.* 1991; Nagpal *et al.* 1992a). The murine RAR-γ gene appears to encode at least two protein isoforms (mRAR-γ1 and mRAR-γ2), which also differ only in their A domains (Giguére *et al.* 1990; Kastner *et al.* 1990). These are encoded by mRNAs generated by alternative splicing of the primary mRAR-γ transcript. There is no evidence that the RAR-γ gene has more than one promoter. Whilst the mRNAs encoding the RAR protein isoforms have been characterized in most detail in the mouse, it is likely that the human RAR genes encode a similar range of proteins (Krust *et al.* 1989).

Detailed information of this sort is not yet available for the RXR receptors. However, there appear to be at least two species of human RXR-β mRNA, which would encode proteins differing only in their A domains (Fleischhauer *et al.* 1992). In addition, we have found that the chicken RXR-γ gene gives rise to at least two, tissue-specific, mRNAs. These mRNAs would encode RXR-γ proteins that differ from each other in their A domains (E. Seleiro, D. Darling and P. Brickell, unpublished data).

## Functional properties of the RARs and RXRs

### *DNA-binding*

The DNA-binding C domain of each of the RARs and the RXRs consists of 66 amino acids which form two cysteine-type zinc fingers. It has been shown for other members of the steroid/thyroid hormone receptor superfamily that the N-terminal finger is involved directly in DNA-binding and that its precise structure is important for controlling the specificity of binding. The second finger is thought to mediate receptor dimerization and thus to control both the specificity and stability of DNA-binding (for review see Berg 1989).

The transcription of a number of genes has been shown to respond to retinoids when cells are treated in culture. Of particular interest is the finding that retinoic acid can induce transcription of the RAR-α and RAR-β genes themselves. For example, treatment of murine F9 embryonal carcinoma cells with all-*trans*-retinoic acid, under conditions that induce them to differentiate into parietal endoderm cells, leads to a rapid increase in the levels of Hox 1.6 mRNA (Gudas 1991), RAR-α2 mRNA (Leroy *et al.* 1991b), RAR-β1 mRNA, RAR-β2 mRNA and RAR-β3 mRNA (Zelent *et al.* 1991). There is also a rapid decrease in levels of REX1 mRNA and a slower increase in levels of Type IV collagen and laminin B1 chain (Gudas 1991). In some cases these changes in gene expression have been shown to result from a direct effect of retinoic acid upon the rate of transcription and have prompted a search for RAREs in the promoter regions of the regulated genes. A list of RAREs and the genes in which they have been found is given in Figure 3. Fragments of DNA that contain these sequences confer retinoic acid responsiveness to heterologous promoters and are able to bind receptor proteins, as demonstrated by gel shift assays (see references in legend to Figure 3).

A common feature of RAREs (Figure 3) is the presence of the core sequence AGGTCA, or of a closely related sequence. This sequence is believed to represent a minimal site for the binding of one receptor molecule, so that the efficient binding of receptor dimers requires the presence of two such core sequences (Beato 1989). The core sequences may be present in the same or opposite orientation with respect to each other. The orientation of the core sequences, and the spacing between them, affects the affinity of receptor binding (Näär *et al.* 1991; Umesono *et al.* 1991). For example, RARs bind more strongly to directly repeated core sequences separated by five base pairs (DR5 motifs) than to directly repeated core sequences separated by three or four base pairs (DR3 and DR4 motifs). Mangelsdorf *et al.* (1991) have shown that the promoter of the rat CRBP-II gene contains a response element that mediates transcriptional transactivation by RXRs but not by RARs. This RXRE contains directly repeated core sequences separated by one base pair (Figure 3), and there is evidence that RXRs may have a general preference for such DR1 repeats (Mangelsdorf *et al.* 1991).

Work on the laminin B1 gene promoter has shown that RARs can bind to RAREs both in the presence and absence of retinoic acid (Vasios *et al.* 1989). The RARs therefore resemble the thyroid hormone receptors, which can bind to thyroid response elements (TREs) in the presence or absence of thyroid hormone, but which activate transcription only in the presence of ligand (Damm *et al.* 1989; Graupner *et al.* 1989).

### *Ligand-binding*

The ligand-binding E domains of the members of the RARs and RXRs have relative molecular mass of approximately 25000. They contain large numbers of hydrophobic residues and its has been suggested that these form a hydrophobic pocket into which the ligand

| Gene | Sequence of retinoic acid response element |
|---|---|
| ADH3 | TGAACTCTGAATGACCC |
| CRBP II | GCTGTCACAGGTCACAGGTCACAGGTCACAGTTCA |
| FH | AGGTCACTGACAGGGCA |
| laminin B1 | TGACCCTTTTTCTAAGGGCTTAACCTAGCTCACCT |
| PEPCK | CTCATGACCTTTGGCCGTGGGA |
| mRAR-α2 | AGTTCAGCAAGAGTTCA |
| mRAR-β2 | GGTTCACCGAAAGTTCA |
| hRAR-β | GGTTCACCGAAAGTTCA |

**Figure 3.** Retinoic acid response elements. ADH3, human alcohol dehydrogenase gene (Duester *et al.* 1991); CRBP II, rat cellular retinol-binding protein II gene (Mangelsdorf *et al.* 1991); FH, mouse complement factor H gene (Muñoz-Cañoves *et al.* 1990); mouse laminin B1 gene (Vasios *et al.* 1989; Glass *et al.* 1990); PEPCK, phosphoenolpyruvate carboxykinase (Lucas *et al.* 1991); mRAR-α2, α2 promoter of mouse RAR-α gene (Leroy *et al.* 1991a, 1991b); mRAR-β2, β2 promoter of mouse RAR-β gene (Sucov *et al.* 1990); hRAR-β, human RAR-β gene (de Thé *et al.* 1990b). Each core element is underlined and its orientation with respect to the gene promoter is shown with a half-arrowhead. The consensus core sequence is: 5′-*A/G G T/G T C A*-3′ (or 5′-*T G A A/C C C/T*-3′ in the opposite orientation).

can fit (Evans 1988; Green & Chambon 1988). The RAR and RXR classes of receptor share no significant amino acid identity in their ligand-binding domains and, consequently, have different retinoid-binding specificities. Two naturally occurring retinoids, all-*trans*-retinoic acid and 9-*cis*-retinoic acid (Figure 1) have been shown to bind to the RARs with high affinity, whilst the RXRs exhibit high affinity binding to 9-*cis*-retinoic acid but not to all-*trans*-retinoic acid (Levin *et al.* 1992; Heyman *et al.* 1992). It is likely that other naturally occurring ligands for these receptors remain to be discovered (Heyman *et al.* 1992; Eager *et al.* 1992).

The characteristics of the RAR ligand-binding domains have been studied more extensively than those of the RXRs. The affinities of the isolated E/F domains of RAR-α and RAR-β for all-*trans*-retinoic acid are identical to those of the intact receptors, indicating that ligand binding to the E domain is independent of the A-D domains (Crettaz *et al.* 1990). The amino acid sequence identity of the E domains of the RAR-α, RAR-β and RAR-γ proteins is high (85–90%), but the differences suggest that these proteins may have different affinities for all-*trans*-retinoic acid. A number of groups have directly determined the affinities of the RARs for all-*trans*-retinoic acid. For example, Crettaz *et al.* (1990) expressed RAR-α and RAR-β proteins in *E. coli* and, using a competition assay, determined $K_d$ values for all-*trans*-retinoic acid binding of 6 nM and 8 nM respectively. There is accumulating evidence that it will be possible to design synthetic retinoids that selectively activate RAR-α, RAR-β or RAR-γ (Martin *et al.* 1992; Apfel *et al.* 1992). Such compounds might be pharmacologically potent, whilst lacking some of the teratogenic side-effects of the retinoids currently used in therapy.

### *Transactivation of transcription*

Transactivation of transcription from target genes clearly requires functional DNA binding and ligand binding domains. However, other members of the steroid/thyroid hormone nuclear receptor superfamily contain two additional regions that can influence the transcription of target genes. These are the AF-1 region, which is located within the N-terminal A and B domains, and the AF-2 region, which is located within the E domain (for reviews see Green & Chambon 1988; Gronemeyer 1991). These regions can enhance transcriptional transactivation in certain cell types and from certain promoters, presumably by interacting with other proteins in the cell. As discussed above, the RAR protein isoforms

encoded by the RAR genes all differ in their A domains. It therefore seems likely that each of the RAR proteins will be found to differ in the efficiency with which they regulate transcription from particular target gene promoters. The more subtle differences between the amino acid sequences of the B domains of the RAR-$\alpha$, RAR-$\beta$ and RAR-$\gamma$ proteins are likely to have a similar effect, as are the subtle differences between the E domains of these molecules. For the same reasons, it is also likely that the range of RXR proteins will have transcription activation characteristics that differ from each other and from the RARs.

To test these ideas, Nagpal *et al.* (1992b) assayed the ability of each of the known RAR and RXR proteins, and of deletion mutants and chimaeric receptors, to transactivate transcription from a range of naturally occurring and synthetic RAREs. They found that each of the known RAR and RXR proteins contains a C-terminal AF-2 region that activates transcription in a ligand inducible and promoter context dependent fashion. Moreover, the AF-2 region of each RAR and RXR protein was found to be unique in its pattern of activity on the RAREs tested. The N-terminal A and B domains of all the RARs and RXRs were found to contain a region that modulates transcriptional activation by the AF-2 region. In chimaeric receptors, the effect of the N-terminal modulatory region on transcription activation varied according to the origin of the AF-2 region to which it was connected and according to the context of the promoter. There is as yet no evidence that the N-terminal modulatory region is able to activate transcription by itself, and it differs in this respect from the AF-1 region found in some other members of the steroid/thyroid nuclear hormone receptor superfamily.

In summary, these data support the idea that the different RAR and RXR proteins regulate different sets of retinoid responsive genes.

### *Dimerization of retinoid receptors*

By analogy with other members of the steroid/thyroid hormone receptor superfamily, it was expected that the retinoid receptors would bind to DNA as dimers, formed by an interaction between sequences within the E domain (Forman & Samuels, 1990). However, homodimers of RAR protein chains bind very poorly to RAREs. A series of recent reports have shown that in order to bind to RAREs with high affinity, RARs must form heterodimers with RXRs (Yu *et al.* 1991; Kliewer *et al.* 1992; Leid *et al.* 1992; Zhang *et al.* 1992a). Such heterodimers bind to RAREs in the absence of ligand and activate transcription in the presence of ligand.

Whilst RARs require RXRs in order to function, the RXRs appear to be able to function independently. Thus, in the presence of 9-*cis*-retinoic acid, RXRs form homodimers that bind strongly to RXREs (Zhang *et al.* 1992b). Changes in the levels of 9-*cis*-retinoic acid within a cell could shift the equilibrium between RAR-RXR heterodimers and RXR-RXR homodimers and thus alter the pattern of target gene activation that results.

### *Interactions between retinoid receptors and other nuclear receptors*

Thyroid hormone nuclear receptors (TRs) and vitamin $D_3$ receptors (VDRs) are structurally more similar to the RARs and RXRs than to other members of the steroid/thyroid hormone receptor superfamily. TRs transactivate transcription in response to thyroid hormone from promoters that contain thyroid response elements (TREs), whilst VDRs transactivate transcription in response to vitamin $D_3$ from promoters that contain vitamin $D_3$ response elements (VDREs). RXR proteins are able to form heterodimers with TRs, increasing both the affinity of DNA-binding and the efficiency of thyroid hormone dependent transcriptional transactivation from promoters containing TREs (Yu *et al.* 1991; Kliewer *et al.* 1992; Leid *et al.* 1992; Zhang *et al.* 1992a). RXRs can also form heterodimers with VDRs, increasing the affinity of DNA binding and the efficiency of vitamin-$D_3$ dependent transcriptional transactivation from promoters containing VDREs (Yu *et al.* 1991; Kliewer *et al.* 1992).

A subset of TREs, including that in the rat growth hormone gene promoter but not that in the rat $\alpha$-myosin heavy chain gene promoter, are also responsive to retinoic acid. Activation of such TREs is enhanced when retinoic acid and thyroid hormone are applied together, and this is thought to result from heterodimer formation between RARs and TRs (Glass *et al.* 1989). Ligand-activated TRs and RARs have also been shown to inhibit transcription of the epidermal growth factor receptor and c-*erb*B2/*neu* genes, in a cooperative manner (Hudson *et al.* 1990). The interaction of RARs and TRs in both activation and inhibition of transcription, through TREs, indicates that retinoic acid and thyroid hormone control the expression of overlapping networks of genes.

## RARE-independent regulation of promoters by RA

Retinoids can down-regulate the expression of a number of metalloprotease genes, including those encoding stromelysin (Nicholson *et al.* 1990) and collagenase (Lafyatis *et al.* 1990). This is thought to account for the potent anti-arthritic activity of some retinoids and for

their efficacy in preventing the spread of some tumours (see below). RARs have been shown to be involved in mediating this negative regulation, but through a pathway that does not involve interaction with a RARE. Rather, the effect is mediated through an AP-1 transcription factor binding site located in the promoters of the stromelysin and collagenase genes. Promoters containing AP-1 sites can be activated by the Jun and Fos transcription factors. These proteins bind to AP-1 sites as Jun–Jun or Jun–Fos dimers. RARs cannot bind to AP-1 sites. Rather, it appears that RARs can form complexes with Jun–Jun and Jun–Fos dimers and inhibit their binding to AP-1 sites. Similarly, whilst Jun–Jun and Jun–Fos dimers cannot bind to RAREs, the formation of complexes with RARs can inhibit retinoic acid dependent transcription from RARE-containing promoters (Pfahl *et al.* 1992).

## Expression patterns of the RAR genes

Extensive in-situ hybridization studies of the distribution of murine and chicken RAR and RXR gene transcripts have been performed. It has proved difficult to raise specific antibodies to RARs and RXRs that stain tissue sections satisfactorily and so no immunocytochemical data about protein distribution are yet available.

The RAR-$\alpha$, RAR-$\beta$ and RAR-$\gamma$ genes are differentially expressed in adult mouse tissues , as indicated in Table 1 (for a review see Ruberte *et al.* 1991). The three genes also have complex patterns of expression in mouse and chick embryos, which change as the embryo develops (for reviews see Ruberte *et al.* 1991; Rowe *et al.* 1992). Of particular relevance to human disease, as discussed below, is the finding that RAR-$\alpha$ mRNA is the predominant RAR mRNA in haematopoietic cells. The apparent role of retinoids in epithelial development and maintenance and the use of retinoids to treat some skin conditions has prompted interest in the pattern of RAR gene expression in the skin and other epithelia. RAR-$\gamma$1 is the predominant species expressed in mouse skin, with low levels of RAR-$\alpha$ also being present. RAR-$\beta$ gene expression has not been detected in mouse skin (for a review see Darmon 1991).

The RXR-$\alpha$ and RXR-$\beta$ genes are expressed in a broad range of tissues during mouse embryogenesis (Mangelsdorf *et al.* 1992), whilst the RXR-$\gamma$ gene has a more restricted pattern of expression in mouse (Mangelsdorf *et al.* 1992), rat (P. Georgiades and P. Brickell, unpublished data) and chick (Rowe *et al.* 1991) embryos. In the adult, low level RXR-$\beta$ gene expression remains widespread, with RXR-$\alpha$ and RXR-$\gamma$ gene expression being more restricted (Mangelsdorf *et al.* 1992; E. Seleiro and P. Brickell, unpublished data).

## RARs in oncogenesis

### *Retinoids and tumour cells*

Retinoids have been shown to regulate the growth and differentiation of a range of cell types, including malignant cells. For example, RAR-$\alpha$ mediates the retinoic acid induced differentiation of the human promyelocytic leukaemia cell line HL60 (Collins *et al.* 1990). This is thought to reflect a role for retinoids in normal myeloid cell differentiation. Retinoids can also induce differentiation of leukaemic cells in patients with acute promyelocytic leukaemia (APL) and all-*trans*-retinoic acid has been used therapeutically to induce complete remission in APL patients (Warrell *et al.* 1991). Similarly, 13-*cis*-retinoic acid has been shown to be effective for treating oral leukoplakia (Hong *et al.* 1986), for preventing second primary tumours in patients with squamous cell carcinoma of the head and neck (Hong *et al.* 1990), for preventing skin tumours in patients with xeroderma pigmentosum (Kraemer *et al.* 1988) and, in combination with interferon-$\alpha_{2a}$, for treating squamous cell carcinomas of the cervix and of the skin (Lippman *et al.* 1992).

### *RAR-$\alpha$ and acute promyelocytic leukaemia (APL)*

In the case of APL, RAR-$\alpha$ has been implicated in the neoplastic process itself. APL is associated with a specific chromosomal abnormality, t(15;17) (q22;q12–21), in which portions of the long arms of chromosomes 15 and 17 are swapped. This balanced translocation involves the RAR-$\alpha$ gene, which lies on chromosome 17, and a previously uncharacterized gene (originally named *myl* but now renamed PML), which lies on chromosome 15 (Borrow *et al.* 1990; de Thé *et al.* 1990a; Alcalay *et al.* 1991). The predicted protein product of the normal PML gene contains a cysteine-rich motif that is present in a number of DNA-binding proteins and that has been proposed to form a novel type of zinc finger structure (Kakizuka *et al.* 1991; de Thé *et al.* 1991). It also contains an amino-terminal proline-rich region that resembles the transcription activation domain of some transcription factors (Kakizuka *et al.* 1991; de Thé *et al.* 1991). It has therefore been suggested that the PML gene encodes a transcription factor (Kakizuka *et al.* 1991; de Thé *et al.* 1991).

When a number of cases of APL were compared, the breakpoints on chromosome 17 were found to cluster within the first intron of the RAR-$\alpha$ gene, between the

exons encoding the A and B domains of RAR-α. The t(15;17) translocation results in the fusion of RAR-α gene sequences downstream of this point, to the end of the PML gene. Since the PML and RAR-α genes are normally transcribed in the same direction, the fused PML-RAR-α gene can direct the synthesis of a PML-RAR-α fusion transcript that encodes a PML-RAR-α fusion protein (Figure 4). In transient expression assays, the PML-RAR-α fusion protein is able to activate transcription from most promoters containing an RARE, in response to treatment with retinoic acid. However, the activity of the fusion protein differs from that of wild-type RAR-α when tested in different cell types and with different RARE-containing promoters (Kakizuka *et al.* 1991; de Thé *et al.* 1991).

The role of the PML-RAR-α fusion protein in APL remains unclear. One possibility is that the fusion protein exerts a dominant negative effect upon RAR-α and so inhibits the activation of RAR-α responsive genes whose expression is required for promyelocyte differentiation. The fact that the fusion protein is able to mediate retinoic acid dependent activation of transcription from most RARE-containing promoters, and in most cell lines, argues against this possibility. However, there is evidence that the fusion protein can suppress the activity of RAR-α on some promoters, and it is possible that such promoters are associated with genes that are important in promyelocyte differentiation (de Thé *et al.* 1991; Kakizuka *et al.* 1991). A second possibility is that the PML-RAR-α fusion protein is a dominant negative inhibitor of the normal PML protein. This model suggests that the PML protein is normally involved in regulating the expression of genes involved in promyelocyte differentiation and that the fusion protein inhibits this activity, perhaps by forming inactive heterodimers with the PML protein. The model further suggests that activation of the fusion protein, by binding of retinoic acid to the ligand-binding domain of the RAR-α portion of the molecule, reverses this dominant negative effect. This would explain why retinoic acid treatment is so effective in inducing APL cell differentiation, and remission of disease in patients with APL (de Thé *et al.* 1991; Kakizuka *et al.* 1991). In support of this model, a number of transcription factors have been shown to become ligand dependent when fused to the ligand binding domains of members of the steroid/thyroid hormone nuclear receptor superfamily (for references, see Kakizuka *et al.* 1991). In order to test these models, target genes for the PML and PML-RAR-α proteins in myeloid cells will have to be identified.

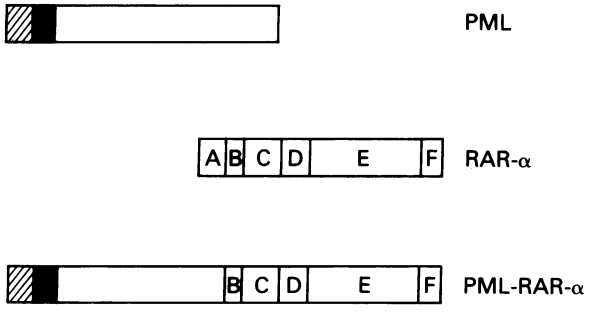


**Figure 4.** Structure of the PML-RAR-α fusion protein encoded by the fusion gene generated by the t(15;17) translocation in acute promyelocytic leukaemia. ▨, Proline-rich region; ■, cysteine-rich region.

### *RAR-β and hepatocellular carcinoma*

The human RAR-β gene was discovered because it was the site of insertion of the hepatitis B virus (HBV) genome in a case of hepatocellular carcinoma (de Thé *et al.* 1987; Benbrook *et al.* 1988). The structure of RAR-β mRNA found in a number of human hepatoma and hepatoma-derived cell lines differs from that found in normal fetal and adult liver (de Thé *et al.* 1987), and it has been suggested that RAR-β could therefore be involved in the development of some human hepatocellular carcinomas.

### *RARs and tumour invasiveness*

Tumour invasiveness and tumour angiogenesis are both believed to involve the degradation of extracellular matrix. In recent years, a number of metalloproteinases with the ability to degrade extracellular matrix and basement membrane components have been discovered (for a review see Matrisian 1990). These include collagenase and stromelysin-3, which is expressed in the stromal cells surrounding the neoplastic cells of invasive breast carcinomas (Basset *et al.* 1990). As described above, retinoic acid can inhibit expression of the stromelysin and collagenase genes. This suggests an explanation for the efficacy of retinoic acid in preventing the spread of some tumours, as discussed above.

## Conclusions

Work performed in the 5 years since the nuclear retinoid receptors were discovered has revealed a signalling system of almost bewildering complexity. This presumably accounts for the range of biological responses that can be elicited by retinoids. Complexity is found at a number of levels in the system.

(1) Tissues contain a number of biologically active retinoids. Their relative levels in a particular cell are likely to be regulated by differences in their catabolism and by differences in their interactions with cellular binding proteins.
(2) There are six retinoid nuclear receptor genes, encoding at least 13 distinct proteins as a result of multiple promoter usage and alternative splicing. These proteins exhibit differences in their affinities for the known biologically active retinoids, and appear to be able to regulate the transcription of different sets of target genes.
(3) Heterodimer formation between RARs and RXRs provides further opportunities for discriminating between target genes.
(4) The RARs and RXRs have complex patterns of expression in embryonic and adult tissues and the particular combination of receptors present in a given cell will be important in determining the outcome of a retinoid signal delivered to that cell.
(5) Opportunities for integrating the response to retinoid signals with the cell's responses to other signals are provided by the ability of the RXRs to form heterodimers with TRs and VDRs, and by the ability of RARs to form inhibitory complexes with Jun–Jun and Jun–Fos dimers. It seems likely that future work will reveal further complex interactions between the retinoid signalling system and other signalling systems.

Much of the above complexity has been revealed by experiments performed in tissue culture or using extracts of cultured cells *in vitro*. The daunting task ahead is to determine which of these interactions are important *in vivo* and to identify the biological systems in which each is relevant. In this way it may eventually be possible to understand how retinoids initiate such a range of biological responses in the normal embryo, in the normal adult, and in disease.

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